Reference: *Biol. Bull.*, 138: 129–137. (April, 1970)

THE EFFECT OF ACTH ON THE SLOUGHING FREQUENCY OF NORMAL AND THYROIDECTOMIZED *ANOLIS CAROLINENSIS* (IGUANIDAE, LACERTILIA)

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Periodic shedding of the surface layer of the skin is a characteristic feature of squamate reptiles that has long been known to be controlled or regulated by hormone action (see reviews: Lynn, 1970; Maderson, Chiu and Phillips, 1970). Since the early work of Drzewicki (1926) and Eggert (1933, 1936) the thyroid has been regarded as the most important endocrine gland involved in this phenomenon. These authors found that in the lizard, *Lacerta,* thyroidectomy results in a cessation of molting and thyroidectomized animals with thyroid tissue implanted in the back muscles are able to carry out at least one or two successful molts. Later studies have confirmed the conclusion that the process of ecdysis in lizards is influenced by the thyroid hormone although there are some species in which thyroidectomy causes only a lengthening of the period between molts rather than a complete inhibition of sloughing (*Hemidactylus,* Noble and Bradley, 1933). For snakes the evidence indicates that the thyroid is also important in the control of the shedding process but acts quite differently; thyroidectomy results in an increased frequency of molting and thyroid administration inhibits it (Schaefer, 1933; Halberkann 1953, 1954; Chiu and Lynn, 1970).

However, it is clear that other hormones than those of the thyroid gland may also be involved in skin shedding in reptiles. It has been shown that administration of adrenocorticotrophic hormone (ACTH) inhibits sloughing in the snake, *Natrix* (Halberkann, 1954; Goslar, 1958) and this hormone has proved similarly effective in hypophysectomized lizards, *Gekko* (Chiu and Phillips, unpublished results). Prolactin administration on the other hand, is reported to increase the sloughing rate in *Anolis* (Maderson and Licht, 1967).

The present study is an examination of the effects of ACTH on the sloughing rate in *Anolis* and on the histological changes in the skin during the sloughing process.

Materials and Methods

A number of shipments of adult *Anolis carolinensis* of both sexes were obtained from May to October, 1968 from a supply company in Louisiana. The specimens were kept in groups of 5 to 10 animals in aquaria measuring $16 \times 10\frac{1}{2} \times 8\frac{1}{2}$ inches at $32 \pm 1°$ C with 7 hours of lighting daily. They were given larvae and adults of

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Tenebrio and the animals were individually observed during each feeding period to make sure that all took food. Water was available at all times. Before any animal was used in the experiment, it was allowed to go through at least two consecutive sloughs so that the length of the pre-experimental sloughing cycle could be ascertained.

The effect of ACTH on shedding was investigated in the following groups: (a) normal, intact, untreated lizards (5 animals); (b) thyroidectomized lizards (9); (c) sham thyroidectomized lizards (5); (d) intact animals receiving intraperitoneal (i.p.) injections of 0.4 I.U. ACTH (Nutritional Biochemical Corp.) in 0.05 ml of a dilute gelatin solution on alternate days (9); (e) intact animals receiving alternate day i.p. injections of 0.05 ml of the gelatin solution used as vehicle in group d (5); (f) thyroidectomized lizards receiving i.p. injections of 0.4 I.U. ACTH in gelatin solution on alternate days (6) and (g) thyroidectomized animals receiving alternate day i.p. injections of 0.05 ml of the gelatin solution used as vehicle for group f (3).

Thyroidectomy was performed under anesthesia with sodium barbital (Nembutal, Abbott) at about 2 mg per 100 g body weight. A transverse incision was made at each side of the ventral surface of the neck anterior to the pectoral girdle, and the two lobes of the thyroid gland were removed. Sham-thyroidectomy was performed in a similar way except that the operation was carried only to point of exposing the gland. The incisions usually healed within a few days.

Thyroidectomy or sham-thyroidectomy and/or administration of ACTH was always performed on the day the animal sloughed. The experiment was terminated at the end of the second slough after treatment in those animals which subsequently sloughed or at the end of at least 60 days in those which did not.

Changes in the epidermis during the sloughing cycle were followed by taking biopsy samples of digits from all animals at roughly weekly intervals. These were fixed in Bouin's fluid, and processed for histological examination using the method described by Maderson and Licht (1967) and Lilywhite and Maderson (1968).

A further set of experiments was performed to assess the effect of ACTH on thyroid activity in the intact animals. A new batch of animals, obtained in February 1969, was kept under identical conditions as the previous lot, and was divided into three groups. (1) normal, intact, untreated controls (5 animals); (2) intact animals receiving i.p. injections of 0.4 I.U. of ACTH in 0.05 ml of gelatin solution daily (5); (3) intact animals receiving i.p. injections of 0.05 ml of the gelatin vehicle daily. The status of the thyroid activity at the end of 7 days was checked by administration of 5 μC of carrier-free I^{131} and measuring thyroidal uptake of the isotope during the subsequent 7 days using equipment and methods described elsewhere (Lynn, McCormick and Gregorek, 1965). At the end of the counting period, the animals were sacrificed and the thyroid glands were processed for histological examination.

Results

General observations

The mortality rate of the lizards in the laboratory was high during the pre-experimental sloughing cycle. It was noted that many animals gradually refused

mealworms and partially digested mealworms were excreted. It is probable that keeping *Anolis* at a constant environmental temperature of 32 ± 1° C and/or feeding this lizard with a monotonous diet of mealworms probably has a detrimental effect on the animal. After treatment was started, some experimental animals died within a period of less than 60 days (26 to 56) during which period no sloughs occurred and these, of course, are not represented in the sloughing records presented in Tables I and II. They came from the following groups; sham thyroidectomized of group c (1 animal); thyroidectomized of group b (6); thyroidectomized receiving ACTH of group f (2); intact animals injected with ACTH of group d (4). With the exception of thyroidectomized animals receiving ACTH (group f) and two thyroidectomized, vehicle controls (group g), all remaining animals sloughed at least once during the experiment. In normal, intact,

TABLE I

The length of the sloughing cycle in Anolis *under various treatments*
Mean ± S. E.

Treatment group	No. of animals	Length of sloughing cycle in days		
		Pre experimental	Post treatment	
			1st	2nd
(a) Intact control	5	18.8 ± 0.6	20.6 ± 2.0 (5)	19.4 ± 2.2 (5)
(b) Thyroidectomized	9	22.3 ± 0.9	51.0 ± 12.1 (4)	48.5 ± 7.5 (2)
(c) Sham − thyroidectomized	5	20.8 ± 0.9	23.0 ± 1.2 (4)	22.0 ± 3.5 (3)
(d) Intact + ACTH	9	21.3 ± 2.5	35.3 ± 4.1 (6)*	34.6 ± 3.0 (5)**
(e) Intact + vehicle	5	20.5 ± 2.3	23.5 ± 1.0 (4)	24.8 ± 0.6 (4)
(f) Thyroidectomized + ACTH	6	32.0 ± 5.7	51.0 (1)	26.0 (1)
(g) Thyroidectomized + vehicle	3	41.3 ± 9.8	35.0 (1)	46.0 (1)

Figures in parentheses refer to number of recorded sloughs after treatment.
* Group d vs. group e, $P < 0.05$.
** Group d vs. group e, $p < 0.02$.

untreated controls (group a), sham-thyroidectomized (group c) and intact, vehicle controls (group e) the process of ecdysis was always complete and required only a few hours. In thyroidectomized lizards and in intact ACTH-treated animals (groups b and d) sloughing was seldom complete. It was usually first indicated by small pieces of shed skin on the digits and the outer epidermal layers of the digit were often shed while the rest of the skin surface showed no sign of shedding at all. The time from this first indication of shedding in the digits to complete removal over the whole body surface might then require 8 to 10 days or might be indefinitely prolonged so that the animals never did completely slough. In these latter animals, no subsequent sloughs were recorded. On the backs of these animals, and of the two thyroidectomized animals receiving vehicle injections, small areas of skin surface were shed. These shed pieces were the result of piling up of the alpha keratin layer, and their occurrence cannot be taken as sloughing records (see below).

Effect on the length of the sloughing cycle (Table I).

Examination of the figures for the pre-experimental cycles given in Table I shows that the cycles for the first five groups (a through e) agree closely while those for the last two groups (f and g) are longer and more variable. This is probably a seasonal difference since experiments a to e were begun in May and June while experiments f and g started in October. However, it will be seen that there is no statistically significant difference in the length of the pre-experimental cycle immediately prior to treatment between various experimental groups (b, d, f) and their controls (c, e, g). Nor is there any significant difference between the lengths of these cycles and those of the experimental cycles in normal, intact, untreated animals (group a), sham-thyroidectomized (group c) and the intact control animals (group e). This indicates that neither sham-thyroidectomy nor vehicle injection have any effect on the length of the sloughing cycle.

A statistically significant increase in the length of the sloughing cycle of about 50% in the ACTH-treated intact animals (group d) was found when compared with the vehicle controls (group e), the normal, untreated controls (group a) and the pre-experimental cycles. A greater increase of the cycle length (about 100%)

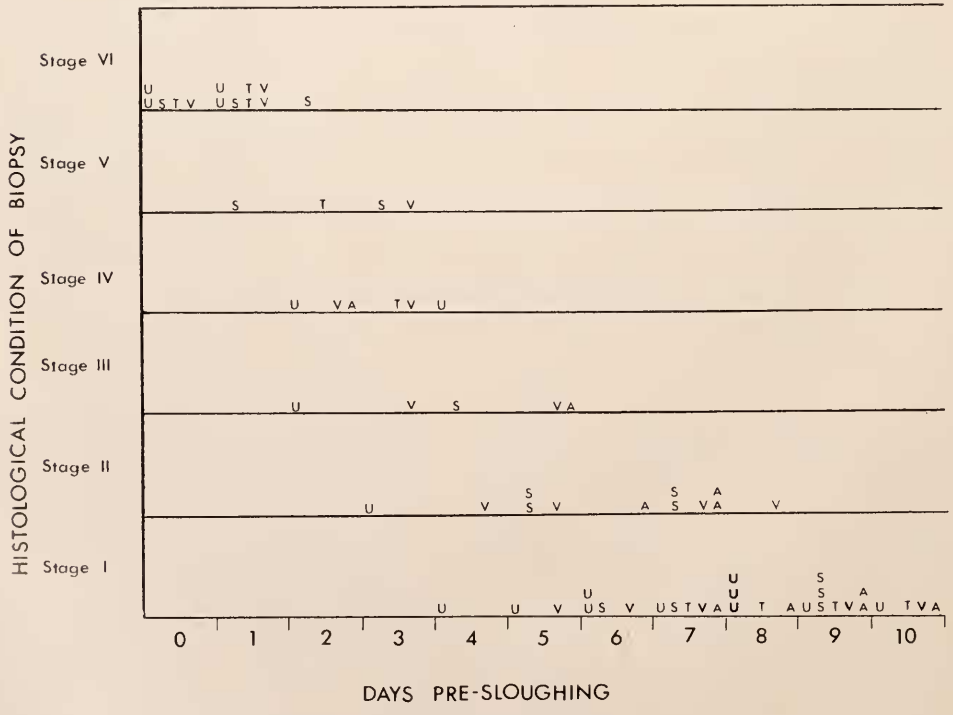

FIGURE 1. Scatter diagram showing the rates of differentiation of the inner epidermal generation during the renewal phase in various groups of *Anolis carolinensis*. U untreated control; S sham-thyroidectomized; T thyroidectomized; V intact plus vehicle; A intact plus ACTH.

was found in four thyroidectomized animals which sloughed (group b) and one thyroidectomized animal receiving vehicle administration (group g) as compared with the sham-operated controls (group c). No slough was noted in ACTH-treated thyroidectomized animals (group f) except in one animal which, on autopsy, was found to have some ectopic thyroid follicles in the mid-ventral line of the neck.

Effect on the epidermis

Histological changes in the epidermis in control animals are similar to those described previously (Maderson and Licht, 1967; Lilywhite and Maderson, 1968) and can be divided into a resting phase and a renewal phase. The lengths of these two phases are comparable among the control groups except in the untreated controls (group a) which appear to have a shorter renewal phase (Fig. 1). In

TABLE II

The percentage uptake of I^{131} *by the thyroid gland in* Anolis
Mean ± S. E.

Treatment group	% uptake of I^{131}						
	1st	2nd	3rd	4th	5th	6th	7th day
(1) Intact control	20.6 ±5.4	30.2 ±6.5	32.8 ±6.6	33.6 ±6.5	31.9 ±6.5	30.4 ±7.3	31.0 ±6.1
(2) Intact +ACTH	16.4 ±3.6	24.0 ±5.1	27.3 ±5.6	28.7 ±5.5	29.8 ±5.6	29.6 ±6.1	28.3 +5.6
(3) Intact +vehicle	17.8 ±6.2	28.5 ±10.4	28.5 ±8.7	28.4 ±8.3	27.2 ±8.6	26.2 ±8.4	25.4 ±7.5

ACTH-treated intact animals (group d) and thyroidectomized animals with or without administration of vehicle (groups g and b) which sloughed, the resting phase is lengthened, and it is indefinitely prolonged in those that failed to slough. Biopsy digit samples taken from those few animals which sloughed indicate that there is no difference in the rate of differentiation of the epidermis in the digit, during the renewal phase as compared with that of the control animals. In both cases the renewal phase occupies about one week. However, since shedding over the general body surface of the ACTH-treated or thyroidectomized animals does not occur synchronously with that on the digits the *total* period of time occupied by the renewal phase in the sloughing cycle is longer as compared with control animals.

In animals that failed to shed, the resting phase is the only phase seen in all biopsies. Samples taken during the first two weeks after ACTH treatment show a typical "resting phase" of the epidermis (Maderson, 1966), in which there is a thin alpha keratin layer lying on top of the stratum germinativum with no cell layers between. Subsequent biopsies, except those taken from thyroidectomized

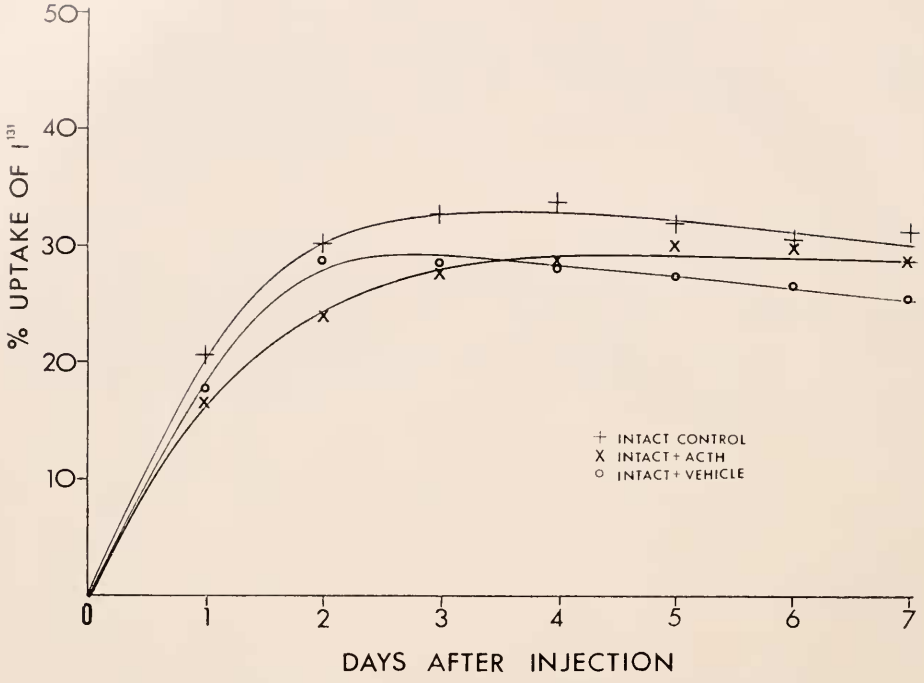

FIGURE 2. The uptake of I^{131} by the thyroid gland after administration of 5 μC of carrier-free I^{131}.

animals injected with ACTH, show a varying number of cell layers above the stratum germinativum and an increase in the thickness of the alpha keratin layer. The stratum germinativum also changes from cuboidal to columnar. In thyroidectomized animals receiving ACTH, no sign of proliferative activity of the germinative layer or increase of the alpha keratin layer can be found.

Effect of ACTH on thyroid activity (Table II and Fig. 2)

There is no significant difference between the percentage uptake of I^{131} in the three groups of intact animals with or without ACTH vehicle (groups 1, 2, and 3). Moreover there is no difference in the histological appearance of the thyroid gland in these animals. This finding suggests that ACTH probably has no influence on thyroid activity in the lizard.

DISCUSSION

It is well known that the thyroid hormones play an important part in the control of ecdysis in lizards. Removal of the thyroid results in complete inhibition of sloughing in the lacertid lizard *Lacerta* (Sembrat and Drzewicki, 1936; Eggert, 1936). However in gekkonids (*Hemidactylus,* Noble and Bradley, 1933; *Gekko,* Chiu *et al.* 1967) thyroidectomy causes only a decrease in sloughing frequency. In

Anolis, an iguanid, earlier reports indicate that thyroid removal or administration of thyroid inhibiting drugs results only in lengthening the sloughing cycle rather than preventing it (Ratzersdorfer *et al.*, 1949; Adams and Craig, 1951). In the present study it was found that half of the thyroidectomized animals showed a decrease in sloughing frequency while the other half failed to shed at all during the period of observation. It seems probable that these diverse results may indicate that, while thyroid function may be the primary controlling agent in skin shedding in lizards, it is not the only factor involved. Several recent studies indicate the significance of other hormonal factors. For example, the hypophysis has been shown to have a role in the sloughing process in lizards and it has been suggested that hypophyseal hormones may have a direct effect on the epidermis (Maderson and Licht, 1967; Chiu and Phillips, unpublished results). Gonadotropins do not influence sloughing in *Anolis* but prolactin causes an increase in sloughing rate (Maderson and Licht, 1967). ACTH has been found to inhibit skin shedding in hypophysectomized *Gekko* (Chiu and Phillips, unpublished results) and the present study shows that administration of ACTH lengthens the sloughing cycle in both normal and thyroidectomized *Anolis.* Furthermore, that this inhibitory action of ACTH is a direct one rather than one intermediated by the thyroid gland is shown by the second series of experiments which indicate that this hormone has no effect upon the thyroidal uptake of radioiodine or upon thyroid histology. It is also unlikely that the effect of ACTH upon ecdysis is mediated through the adrenal gland for it has been demonstrated that administration of adrenocorticoids has no effect on sloughing in squamate reptiles (Halberkann, 1954; Chiu and Phillips, unpublished results).

The effects of various hormones on epidermal changes during the sloughing cycle have been investigated in a number of lizards (Eggert, 1936; Sembrat and Drzewicki, 1936; Adams and Craig, 1951; Chiu *et al.*, 1967; Maderson and Licht, 1967; Chiu and Phillips, unpublished results). While no abnormality in epidermal histology has been noted in *Gekko* under various endocrine manipulations (Chiu *et al.*, 1967; Chiu and Phillips, unpublished results), or in *Anolis* under prolactin administration (Maderson and Licht, 1967), an increase in the thickness of the keratin layer after thyroidectomy (*i.e.*, hyperkeratosis) has been reported in *Lacerta* (Eggert, 1936; Sembrat and Drzewicki, 1936) and in *Anolis* (Adams and Craig, 1951). In the present study such an increase in thickness of the alpha keratin layer was noted about 40 days after sloughing in thyroidectomized animals that have greatly protracted cycles and in those that do not slough at all. This finding confirms Adams and Craig's (1951) study on *Anolis.* However we have also found this phenomenon in ACTH-treated intact animals. We would suggest hyperkeratosis of the alpha keratin layer is probably a general feature in any protracted cycle in *Anolis.* It has been seen that proliferation of the stratum germinativum continues in thyroidectomized animals and also in non-thyroidectomized animals treated with ACTH. A delay in the appearance of the renewal phase after thyroidectomy and subsequent keratinization of the newly formed cells would result in an increase in thickness of the alpha keratin layer. It is understandable that no increase of keratin layers is observed in thyroidectomized animals receiving ACTH since no proliferative activity of the germinative layer of the epidermis is evident in these animals. We may conclude that thyroidectomy delays differenti-

ation of the epidermal cell layers, and that ACTH has an inhibitory effect on cellular proliferation in the epidermis.

We are grateful to Pauline Chiu for her technical assistance.

Summary

The effect of thyroidectomy and administration of ACTH (0.4 I.U. on alternate days) on sloughing performance in normal and thyroidectomized *Anolis* has been investigated. Either thyroidectomy or injection of ACTH decreases the frequency of sloughing, but does not inhibit it. ACTH administration and thyroidectomy together prevent sloughing. Histological study of the skin shows that the resting phase of the sloughing cycle is lengthened in thyroidectomized animals and in intact animals receiving ACTH injections, and is indefinitely prolonged in thyroidectomized animals injected with ACTH. Hyperkeratosis (increase in the thickness of the keratin layer) is observed in all animals with a protracted cycle, except in thyroidectomized animals with ACTH administration. Since there is no difference in the I^{131} uptake or in the histological appearance of the thyroid gland following ACTH treatment, it is suggested that ACTH influences the sloughing cycle independently of the thyroid gland probably through a direct inhibitory effect on cellular proliferation.

LITERATURE CITED

Adams, A. E., and M. Craig, 1951. The effects of antithyroid compounds on the adult lizard, *Anolis carolinensis*. *J. Exp. Zool.*, **117**: 287–315.

Chiu, K. W., and W. G. Lynn, 1970. The role of the thyroid in skin-shedding in the shovel-nosed snake, *Chionactis occipitalis*. *Gen. Comp. Endocrinol.*, in press.

Chiu, K. W., J. G. Phillips and P. F. A. Maderson, 1967. The role of the thyroid in the control of the sloughing cycle in the Tokay (*Gekko gecko*, Lacertilia). *J. Endocrinol.*, **39**: 463–472.

Drzewicki, S., 1926. Influence de l'extirpation de la glande thyroide sur la mue du lézard (*Lacerta agilis* L.). *C. R. Séanc. Soc. Biol.* **95**: 893–895.

Eggert, B., 1933. Ueber die histologischem und physiologischen Beziehungen zwischen Schilddrüse und Häutung bei den einheimischen Eideschen. *Zool. Anz.*, **105**: 1–9.

Eggert, B., 1936. Zur Morphologie und Physiologie der Eideschsenschilddrüse. 3. Über die nach entfernung der Schilddrüse auftretenden allgemeinen Ausfallserscheinungen und über die Bedeutung der Schilddrüse für die Häutung und für die Kaltestarre. *Z. Wiss. Zool.*, **148**: 221–260.

Goslar, H. G., 1958. Die Reptilienhaut als endokrines Testobjekt. *Endokrinologie*, **36**: 279–286.

Halberkann, J., 1953. Untersuchungen zur Beeinflussung des Häutungszyklus der Ringelnatter durch Thyroxin. *Arch. Dermatol. Syphilol.* **197**: 37–41.

Halberkann, J., 1954. Zur hormonalen Beeinflussung des Häutungszyklus der Ringelnatter. *Z. Naturforsch.*, **96**: 77–80.

Lilywhite, H. G., and P. F. A. Maderson, 1968. Histological changes in the epidermis of the subdigital lamellae of *Anolis carolinensis* during the shedding cycle. *J. Morphol.*, **125**: 379–402.

Lynn, W. G., 1970. The thyroid. *In:* A. D'A Bellairs, C. Gans and E. Williams, eds., *The Biology of the Reptilia*. Academic Press, New York, in press.

Lynn, W. G., J. J. McCormick and J. C. Gregorek, 1965. Environmental Temperature and thyroid function in the lizard, *Anolis carolinensis*. *Gen. Comp. Endocrinol.*, **5**: 587–595.

MADERSON, P. F. A., 1966. Histological changes in the epidermis of the Tokay (*Gekko gecko*) during the sloughing cycle. *J. Morphol.*, **119**: 39–50.

MADERSON, P. F. A., K. W. CHIU AND J. G. PHILLIPS, 1970. Endocrine-epidermal relationships in squamate reptiles. *Mem. Soc. Endocrinol.*, in press.

MADERSON, P. F. A., AND P. LICHT, 1967. Epidermal morphology and sloughing frequency in normal and prolactin treated *Anolis carolinensis* (Iguanidae, Lacertilia). *J. Morphol.*, **123**: 157–171.

NOBLE, G. K., AND H. T. BRADLEY, 1933. The relation of the thyroid and the hypophysis to the molting process in the lizard, *Hemidactylus brookii*. *Biol. Bull.*, **64**: 289–298.

RATZERSDORFER, C., A. S. GORDON AND H. A. CHARIPPER, 1949. The effects of thiourea on the thyroid gland and molting behavior of the lizard, *Anolis carolinensis*. *J. Exp. Zool.*, **112**: 13–27.

SCHAEFER, W. H., 1933. Hypophysectomy and thyroidectomy of snakes. *Proc. Soc. Exp. Biol. Med.*, **30**: 1363–1365.

SEMBRAT, K., AND S. DRZEWICKI, 1936. The influence of the selachian thyroid upon the molting process of the lizards, with some remarks on the skin, the eyes, and the ultimobranchial body of the thyroidectomized lizards. *Zool. Pol.* **1**: 119–169.